Apparent Antagonistic Effects between 3,3',4,4',5-Pentachlorobiphenyl and 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin on Hepatic Retinoid Loss in the Rat: Possible Involvement of CYP1A2.

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Abstract

Female Sprague-Dawley rats were fed diets containing 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and/or 3,3',4,4',5-pentachlorobiphenyl (PCB 126) in various concentrations for three months. Hepatic retinoid loss, CYP1A1 induction and CYP1A2 induction were markedly influenced at 0.4 µg/kg TCDD or 7 µg/kg PCB 126. Co-administration of PCB 126 and 0.4 µg/kg TCDD resulted in an antagonistic effect on hepatic retinoid loss. Co-administration of PCB 126 and 5 µg/kg TCDD gave an antagonistic effect on CYP1A2 activity. No interactive effects were found on CYP1A1 activity. PCB 126 liver retention was lower with co-administration of TCDD. A possible role of CYP1A2 in hepatic retinoid loss is hypothesized.

Introduction

Recently, we have reported that 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) have resulted in an extensive hepatic retinoid loss in a 13-week feeding study[1]. This effect has been reported frequently for TCDD and various co-planar polychlorinated- and polybrominatedbiphenyl congeners, in contrast to PCB 153. Based on these and other observations, the Ah-receptor may be involved in this hepatic retinoid effect[2]. Moreover, it has been shown that *in vitro* purified rabbit CYP1A2 and CYP2B4 could hydrolyse retinoic acid, retinol, and retinal, mainly at the 4-position[3].

In this study we investigated the effects on CYP1A1 and CYP1A2 activities, and

the loss of hepatic retinoids in the rat after subchronic exposure to PCB 126, TCDD, or combinations of both compounds.

Methods

Chemicals: 3,3',4,4',5-Pentachlorobiphenyl (PCB 126) was from Schmidt B.V. (Amsterdam, The Netherlands). A small amount of [^{3}H]TCDD (obtained from Givaudan, Switzerland) was added to TCDD originating from Dow Chemical (Midland, Mi USA).

Animal treatment: Female Sprague-Dawley rats (Iva: S/V 50 (SD)), 8 animals per group, starting weight about 150 grams, were fed on experimental diets for 13 weeks. The diets, pulverized feed (Nafag 890), contained 0, 7, 50, or 180 µgPCB 126/kg diet, 0.4 or 5 µgTCDD/kg diet, or combinations of both. Supplementation of the diets was performed according to Pluess *et al*[4]. Food consumption was recorded twice a week.

Retinoid measurements: Liver retinoids were analysed according to Brouwer *et al*[5].

Enzyme induction: Microsomal CYP1A1 activity was determined as 7-ethoxyresorufin-O-deethylation (EROD) by using the method of Burke *et al*[6]. Microsomal CYP1A2 activity was measured as the 4-hydroxylation of acetanilide (4 OH-AA) according to Liu *et al*[7]. Protein levels were analysed according to Bradford[8].

Residue analyses: Liver PCB 126 levels were analysed by GC-ECD after a clean up procedure described by De Jongh *et al.*[9], using the first column reported. Residues of TCDD in liver were determined as described earlier[10].

Statistics: Data were analysed for differences to control by using ANOVA (least significant difference, LSD test).

Results and discussion

Table 1 summarizes the effects of PCB 126 and/or TCDD on CYP1A1 activity, measured as EROD, CYP1A2 activity, measured as acetanilide hydroxylation (4 OH-AA), and hepatic retinoid levels.

Obviously, CYP1A1 and CYP1A2 activities were markedly induced by 7 µg/kg of PCB 126, i.e. 32 and 2.9 times control levels, respectively. At the same dose, the levels of retinol and retinylpalmitate were dropped to 41% and 22% of control values, respectively. Higher doses of either PCB 126 or TCDD almost completely eliminated hepatic retinoid levels.

Co-administration of 0.4 µg/kg TCDD and PCB 126, at all doses tested, resulted in less reduction in hepatic retinol than following single compound treatment. Co-administration of 5 µg/kg TCDD and PCB 126 induced a nearly complete loss in hepatic retinoid levels.

Maximum CYP1A1 (EROD) and CYP1A2 (4 OH-AA) activities were reached at the 50 µg/kg dose level of PCB 126. Co-administration of 50 µg/kg PCB 126 and 5 µg/kg TCDD did not further increase EROD activity. In contrast, CYP1A2 activity was PCB 126 dose dependently reduced by co-administration with 5 µg/kg TCDD. The liver retention of PCB 126 in co-administration with 0.4 or 5 µg/kg TCDD was reduced to about 70% of levels in the PCB 126 groups only. This may be a consequence of

Table 1. CYP1A1 (EROD), CYP1A2 (4 OH-AA), and hepatic retinoid concentrations after 13 weeks on diets containing PCB 126 (mean ± SE, n=8).

Dose PCB 126 (µg/kg diet)	PCB in liver % of dose	Dose TCDD (µg/kg diet)	TCDD in liver (% of dose)	EROD (nmol/mg.min)	4 OH-AA (µg/mg.min)	Hepatic retinol (mg/g liver)	Hepatic retinyl-palmitate (mg/g liver)
0	0	0	0	0.19 ± 0.05	0.65 ± 0.07	14.9 ± 3.1	472 ± 96
7	24.5 ± 3.4	0	0	6.08 ± 1.50*	1.87 ± 0.10*	6.2 ± 0.6*	103 ± 26*
50	23.5 ± 4.6	0	0	9.11 ± 1.30*	2.33 ± 0.11*	1.5 ± 0.3*	13 ± 2*
180	27.7 ± 4.3	0	0	9.27 ± 2.05*	2.00 ± 0.08*	0.6 ± 0.1*	1 ± 0.3*
0	0	0.4	4.65 ± 0.50	4.10 ± 1.13*	1.61 ± 0.12*	8.2 ± 0.8*	107 ± 27*
7	17.2 ± 3.6	0.4	4.70 ± 1.05	6.71 ± 0.67	2.20 ± 0.13#	10.7 ± 1.1	60 ± 20
50	18.0 ± 4.1	0.4	5.06 ± 0.69	8.81 ± 2.81	2.65 ± 0.22#	4.0 ± 1.1#	23 ± 6#
180	16.1 ± 2.8	0.4	4.57 ± 0.76	10.52 ± 2.41	2.23 ± 0.16#	4.8 ± 0.9#	30 ± 6#
0	0	5	5.95 ± 0.74	9.95 ± 1.05*	2.39 ± 0.19*	2.2 ± 0.3*	22 ± 8*
7	16.1 ± 2.7	5	5.12 ± 0.72	9.35 ± 1.32	2.49 ± 0.15	0.9 ± 0.2$	5 ± 1
50	16.2 ± 5.1	5	5.51 ± 0.60	10.62 ± 1.62	2.16 ± 0.15	1.8 ± 0.7	24 ± 21
180	14.2 ± 3.7	5	5.13 ± 0.83	9.26 ± 2.60	1.74 ± 0.10$	1.4 ± 0.3	5 ± 1

* Significant to control (LSD test, p<0.025)
# Significant to 0.4 µg/kg TCDD (LSD test, p<0.025)
$ Significant to 5 µg/kg TCDD (LSD test, p<0.025)

competition between PCB 126 and TCDD for CYP1A2, whereas strong binding affinities of 2,3,7,8-substituted PCDDs and PCDFs to CYP1A2 has been reported earlier from other studies[12,13].

Antagonistic effects between PCB 126 and TCDD on hepatic retinoid contents occurred at 0.4 µg/kg TCDD. In relation to these observations, CYP450[11] and, moreover, CYP1A2[3] have been reported to be involved in the catabolism of retinol and retinoic acid *in vitro*. However, this does not explain the loss in hepatic retinylpalmitate by TCDD or PCB 126. Since hepatic retinoid depletion seemed a very sensitive effect upon the administered compounds, the involvement of cytochrome P450 in the catabolism of retinoids needs further investigation.

The results from our study suggested that CYP1A2 and not CYP1A1 may possibly be involved in the catabolism of hepatic retinoids *in vivo*.

Acknowledgements

We are greatly indebted to Dr. L. Birnbaum and Dr. M. DeVito for expert help with the set-up of the CYP1A2 assay.

This investigation was subsidized by The Technology Foundation, The Netherlands, grant UDG 99.1869 and The Swiss National Foundation, Switzerland, grant 2-77-330.91.

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